

allowed to evaporate at room temperature to a very small volume and then subjected to infra-red analysis. The presence of two hydroxyl groups adjacent to each other (*o*-position) in the compound was revealed.

A steep rise in optical density of an RNA solution at 260 μ was noted upon the addition of an increasing amount of catechol. This information allows one to conclude that the material extracted from adult insects with water and responsible for giving a high value for free nucleotide concentration when measured at 260 μ , is catechol.

Résumé. A côté de neuf acides aminés un composé polyphénolique est décelé dans l'extrait aqueux de *Tribolium confusum* Duval. Le chromatogramme sur papier traité par $K_3Fe(CN)_6$ et l'analyse I.R. révèlent qu'il s'agit du catéchol.

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Mutant Expression in Inbred Lines and Crosses

When a quantitative character can be scored more than once on single individuals, its phenotypic variance may be regarded as made up of two components, V_c and V_i . V_c refers to the variance common to the repeated scores on an individual while V_i refers to the independent variance of each score alone¹. For a character which can be measured on the right and the left-hand side of an individual, the variance from, say, the left side will be $V_L = V_c + V_i$ and the variance of the sum of the measurements on the two sides will be² $V_{L+R} = 4V_c + 2V_i$.

In genetically homogeneous populations, the common component of variance V_c is attributable to variation in the external environment. The independent component is variously described as 'chance or stochastic variability'³, 'developmental error'⁴, 'developmental noise'⁵, and 'internal accidents in development'⁶, terms in which the authors try to express the organism's inability to realize a complete, bilaterally identical course of development notwithstanding identical genetic and environmental conditions.

It has been shown in *Drosophila* that the relative contributions of the two components of variance differ according to the type of quantitative character. This is further reflected in characteristic differences between inbred lines and crosses with respect both to variance and to the value of the mean, relative to those of the parents. The causes and consequences of such differences are still obscure. We need similar analyses on a greater variety of characters in different environmental situations and in novel genetic conditions as in the expression of mutants of variable expression. There the properties of the system can hardly be attributed to a long established adjustment by natural selection.

The mutant used for this purpose was *cubitus interruptus* dominant (*ci*^D, 4th chromosome) of *Drosophila melanogaster*. In single dose it causes a terminal interruption of the 4th longitudinal vein of the wing. With the help of suitable, marked stocks this mutant has been introduced into several inbred lines so that the variance of expression can be studied in different genetic backgrounds. The lines were derived by long continued brother-sister mating from the Gabarros and Pacific wild stocks. The different inbred lines and crosses between them were reared in $\frac{1}{8}$ l creamers at 25°C and at low densities to eliminate possible effects of crowding. Both wings were scored for expression of *ci*^D on 20 individuals of each sex per culture. Expression is measured as the ratio of the length of the 4th vein present to that of the uninterrupted 3rd vein, both measured from the anterior crossvein^{7,8}.

The results are shown in the Table. Only those lines and crosses are shown in which the entire distribution of expression falls within that part of the scale where expres-

sion is approximately linear in relation to genetic and environmental changes. A description of the various tests and a discussion of this problem has been given earlier⁸.

In all crosses the mean values of the scores in ♀♀ are close to that of the midparent value, thereby providing additional support for the linearity of this part of the scale. With respect to the variance, the independent component V_i is several times larger than the common component V_c . This is in agreement with unpublished experiments, carried out in collaboration with Dr. F. W. ROBERTSON, which showed that considerable changes in body size and developmental time on different chemically defined sterile media were not accompanied by alteration in the average expression of *ci*^D which is therefore insensitive to such gross changes in the environment. Also different levels of crowding and larval growth at successive periods in live yeast cultures did not affect the expression.

In the Table all estimates of V_i and V_c are combined in weighted estimates for all crosses and all inbreds respectively. The values of V_i are remarkably similar, but the V_c value for crosses is smaller. As, however, both V_i and V_c are not homogeneous within inbreds and within crosses (using Bartlett's test $P < 0.05$ for V_i values and

Means and variance components of the expression ratio of *ci*^D in inbred lines and crosses. Explanation in text

Inbred line or cross	mean ♀♀	N	V_{L+R}	$2V_i$	$4V_c$
Gabarros 2	39.8	120	27.32	17.65	9.67
Gabarros 4	41.7	80	32.98	30.63	2.35
Gabarros 6	37.1	160	41.54	22.85	18.69
Pacific 4	60.4	120	36.01	17.03	18.98
Gab. 2 × Gab. 4	41.9	160	20.14	19.35	0.79
Gab. 2 × Pac. 4	51.0	40	44.77	31.54	13.23
Gab. 2 × Pac. 10	49.8	160	29.10	23.35	5.75
Gab. 4 × Pac. 4	52.2	40	35.41	22.68	12.68
Gab. 4 × Pac. 10	53.8	160	34.36	21.10	13.26
Pac. 4 × Pac. 10	58.5	160	40.58	30.31	10.27
Total inbreds		480	35.30	22.55	12.75
Total crosses		720	31.40	23.52	7.88

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$P < 0.001$ for V_c values) a closer examination seems recommendable. Comparison of the V_i values in the crosses and their parent lines shows always a similar or somewhat higher V_i in the crosses. But the situation for V_c is less obvious.

The behaviour of this character recalls that of abdominal chaetae^{1,2,5} but is clearly different from that of body size, in which V_c is by far the larger component which when inbred lines are crossed, declines sharply while the mean increases³. In number of ovarioles, too, V_i is several times larger than V_c ⁴ but in this character crossing of inbred lines led to a clear decline in V_c along with an increase in the mean. The expression of ci^D differs from sternopleural bristles where the relatively high V_i generally decreases when inbred lines are crossed, while V_c is unaltered and the mean is intermediate^{2,6}.

These contrasts underline the dangers inherent in the *a priori* argument that it must be an advantage for a bilaterally symmetrical organism to have its two sides as similar as possible, and that therefore asymmetry in bilateral characters can be used as a measure of general developmental stability or homeostasis¹⁰. Each case must be considered on its own merits.

Development in heterozygotes is supposed to be more efficiently buffered against environmental and genetic differences than is development in inbred lines¹¹. On the basis of this assumption we should expect that the expression of mutants would tend to regress to wild type when inbred lines were crossed. This is not so, either in our experiments or in those of SONDI with ocellless in *Drosophila subobscura*¹². A possible explanation would be that the greater buffering ability of heterozygotes is only

effective for a certain range and variety of disturbances commonly encountered in populations, but not for mutant effects which greatly transgress the normal levels of variation¹³.

Zusammenfassung. In Versuchen mit der Mutante ci^D von *Drosophila melanogaster* wurden die Komponenten der nichtgenetischen Varianz der Merkmalsausprägung bestimmt durch Einkreuzen von ci^D in verschiedene Inzuchtstämme. Die unabhängige Komponente (V_i) für die beiden Flügel ist mehrere Male grösser als die gemeinsame Komponente (V_c). Nach Kreuzung zwischen den Inzuchtstämmen wurde keine Veränderung der V_i -Werte und in den Werten der Merkmalsausprägung keine Annäherung an den normalen Phänotypus festgestellt.

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¹² K. C. SONDI, J. Genet. 57, 193 (1960).

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Gewinnung von Leukocyten aus Rinderblut unter Verwendung von Wasser als Hämolysikum

Im Verlaufe unserer Untersuchungen über Inhaltsstoffe von präparativ gewonnenen eosinophilen Granulocyten des Pferdeblutes¹ erschien es uns wünschenswert, diese Zellen auch aus Rinderblut zu erhalten.

Voraussetzung für die Isolierung von Eosinophilen ist das Vorhandensein einer Leukocytensuspension². Nur aus Pferdeblut lassen sich derartige Suspensionen ohne weiteres gewinnen, da sich die Erythrocyten infolge Geldrollenbildung schneller absetzen als die Leukocyten. Bei der Durchsicht der Literatur nach Methoden der Abtrennung von weissen Blutzellen aus Vollblut³⁻⁷ stellten wir fest, dass dieses Problem noch keine befriedigende Lösung gefunden hat. Am aussichtsreichsten erschien für unsere Arbeiten die unter anderem von POLLI⁸ angewandte Methode, die Senkungsgeschwindigkeit der Erythrocyten durch Zusatz von *Gummi arabicum* zu beschleunigen, um ähnliche Verhältnisse wie beim Pferdeblut zu schaffen. Dieses Verfahren führte jedoch bei Rinderblut nicht zum Ziel. Das veranlasste uns, nach einem anderen Weg zu suchen. Die schliesslich von uns gefundene Methode ist einfach. Sie verlangt keinen besonderen apparativen und zeitlichen Aufwand und ermöglicht es, beliebig grosse Blutmengen zu verarbeiten.

Prinzip. Wir hämolysieren Citratblut mit einer ausreichenden Menge Wasser und stellen unmittelbar anschliessend durch Zusatz einer entsprechend konzentrierten NaCl-Lösung das isotonische Milieu wieder her.

Methode. 1600,0 cm³ Blut werden in 400,0 cm³ 3,8%iger Na-Citratlösung aufgefangen und durch Zusatz von 4 l Wasser zur Hämolyse gebracht (Blut:Wasser-Verhältnis

1:2). Man bringt das Citratblut in einen Kunststoffeimer und giesst aus einem zweiten 4 l Wasser hinzu. Durch mehrmaliges Umschütten von Eimer zu Eimer wird für eine gute Durchmischung gesorgt. Dabei ist entsprechend behutsam vorzugehen, um Schädigungen der weissen Blutzellen und störende übermässige Schaumbildung zu vermeiden. Zur Wiederherstellung physiologischer Verhältnisse wird das hämolysische Blut in einen Eimer mit 1 l 4,5%iger NaCl-Lösung (Stammlösung) eingegossen und wie oben durch Umgiessen von Eimer zu Eimer gut durchmischt. Beide Arbeitsgänge – Hämolyse und Wiedereinstellung der Isotonie – lassen sich leicht in ca. 30 bis 60 sec bewerkstelligen. Auf diese Weise sind die Leukocyten nur kurze Zeit hypotonischen Verhältnissen ausgesetzt. Bei kleineren Blutmengen lässt sich schneller arbeiten. Die angegebene Zeit sollte jedoch nicht unterschritten werden, damit eine ausreichende Hämolyse gesichert ist.

Anstatt mit NaCl-Lösungen bestimmter Konzentration kann man die Isotonie auch mit entsprechend konzentrierten Stammlösungen physiologischer Lösungen wiederherstellen. Diese Stammlösungen müssen die einzelnen

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